

Chapter 25

Selenoproteins Harboring a Split Personality in Both Preventing and Promoting Cancer

Min-Hyuk Yoo, Bradley A. Carlson, Petra A. Tsuji, Ryuta Tobe,
Salvador Naranjo-Suarez, Byeong Jae Lee, Cindy D. Davis,
Vadim N. Gladyshev, and Dolph L. Hatfield

Abstract Selenium has long been known to have a role in preventing cancer, but only in recent years has a deficiency in this element also been shown to function in preventing cancer. Selenoproteins have also been shown to serve as selenium-containing components in cancer prevention, but we are learning that specific members of this protein class can also function in cancer promotion. The involvement of two of these selenoproteins, thioredoxin reductase 1 and the 15 kDa selenoprotein, in promoting cancer is examined in this chapter.

M.-H. Yoo • B.A. Carlson • R. Tobe • S. Naranjo-Suarez • D.L. Hatfield (✉)
Molecular Biology of Selenium Section, Laboratory of Cancer Prevention,
Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda,
MD 20892, USA
e-mail: hatfield@mail.nih.gov

P.A. Tsuji
Molecular Biology of Selenium Section, Laboratory of Cancer Prevention,
Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda,
MD 20892, USA

Cancer Prevention Fellowship Program, Bethesda, MD 20892, USA

B.J. Lee
Department of Biological Sciences, Interdisciplinary Program for Bioinformatics,
Seoul National University, Seoul, South Korea

C.D. Davis
Nutritional Science Research Group, Division of Cancer Prevention,
National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

V.N. Gladyshev
Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Harvard
Medical School, Boston, MA 02115, USA

25.1 Introduction

Selenium has been associated with many health benefits in humans and other mammals that include roles as a cancer chemopreventive agent, a preventive of heart disease, other cardiovascular diseases and muscle disorders, and in mammalian development, slowing the aging process, delaying the onset of AIDS in HIV-positive patients, and inhibiting viral expression [1, 2]. The benefit of selenium in health that has received the most attention by far is its role in preventing cancer [1, 2]. Whether small molecular weight selenium-containing compounds (selenocompounds) or selenium-containing proteins (selenoproteins), or both of these classes of agents, are responsible for the health benefits of this element in cancer has been the subject of considerable debate in the selenium field [3–9]. Evidence supporting both proposals has been reported [3–12], but the emphasis of the research in this area appears to be shifting in favor of the role of selenoproteins (see other chapters in this book) of which there are 25 selenoprotein genes in humans and 24 in rodents [13]. There are, of course, more selenoprotein forms in humans and rodents than genes due to transcription variants, different start signals for initiating selenoprotein synthesis and splicing variants in a single mRNA (e.g., human thioredoxin reductase 1 (TR1) has at least six known transcription variants [14] and glutathione reductase 4 (GPx4) has alternative transcripts with two distinct promoter regions [15]). In addition, multiple Sec UGA codons may occur in a single mRNA that results in both partial termination and partial read-through at UGA codons downstream of the initial selenocysteine (Sec) codon (i.e., selenoprotein P (SelP)). Partial termination of SelP at the second and third Sec codons yields two truncated, likely functional, proteins [16].

Of the many selenoproteins known in mammals [8], there are at least three that have a split personality in that they apparently have roles in both preventing and promoting cancer. These selenoproteins are TR1 and the 15 kDa selenoprotein (Sep15), which are described below, and glutathione peroxidase 2 (GPx2), which is described in Chap. 21.

25.2 TR1

TR1 has long been recognized as one of the major antioxidant and redox regulators in mammalian cells [8, 13], and more recently, as an essential protein in mammalian development [14]. Both the selenium-containing form, wherein the selenium atom occurs at the active site as the amino acid, Sec, and the corresponding cysteine-containing form in which cysteine is inserted in place of Sec, are known. Since a major function of TR1 is to control the redox state of thioredoxin and therefore protect normal and malignant cells from oxidative stress, it would seem that TR1's role in both promoting and preventing cancer may be, at least in part, the same. That is, TR1 prevents oxidative damage in normal cells by helping maintain them in a

healthy state, and regulates redox homeostasis in cancer cells by assisting them in overcoming the burdens of oxidative damage. These plausible roles of TR1 in normal and cancer cells are further discussed below.

There are other activities of TR1 that provide further evidence of its role in normal cells as a cancer preventive agent. For example, this selenoenzyme is known to activate the p53 tumor suppressor and to have other tumor-suppressor activities [17], and is specifically targeted by carcinogenic electrophilic compounds [18, 19]. Thus, TR1 clearly is an anticancer protein.

TR1 also has roles in promoting cancer. This selenoenzyme has been shown by several investigators to be overexpressed in many cancer cell lines and cancers [20–23]. In addition, cancer-related properties of malignant cells have been shown to be altered by specifically targeting and inhibiting TR1 activity by using a variety of anticancer drugs and potent inhibitors [20–25]. Furthermore, rendering cancer cells TR1 deficient has been shown to reverse several of the cancer characteristics [26, 27]. Thus, TR1 clearly is a procancer protein.

A major focus of our laboratories over the past several years has been to elucidate the underlying cellular mechanisms of how TR1 can have a role in preventing cancer and then switch its function to one of promoting cancer [26–28]. We initially targeted the removal of TR1 in a mouse lung cancer cell line, designated LLC1 [26]. More than 90% of TR1 expression was lost in LLC1 cells carrying the knockdown vector compared to control cells carrying the same vector except that it lacked the targeting sequence (Fig. 25.1 a1). As a result, the morphology, expression of two cancer-related mRNAs, *Hfg* and *Opn1*, anchorage-independent growth and other of the cells' malignant properties were altered to become more similar to those of normal cells following the downregulation of TR1 expression [26]. Injection of TR1-deficient LLC1 cells into wild type, genetically compatible mice resulted in a dramatic reduction in tumor development in mice injected subcutaneously (Fig. 25.1 a2) or in lung metastasis in mice injected in the tail vein (Fig. 25.1 b1) compared to wild-type mice injected with the control vector. Tumors arising in the flank were much smaller in size than the corresponding tumors that developed in mice injected with the control vector. Most importantly, the smaller tumors in mice injected with the TR1 knockdown vector had lost the vector (Fig. 25.1 a3) demonstrating that tumor development caused by LLC1 cells is dependent on TR1 expression. Pathological analysis of lungs of mice injected with TR1-deficient cells had no detectable pathological changes, while those injected with the control TR1-sufficient cells showed extensive malignant alterations (Fig. 25.1 b2).

The above studies demonstrate a direct dependency of the malignancy process on an enriched TR1 expression in LLC1 cells. They do not, however, show the underlying mechanism(s) of how TR1 is responsible for promoting and/or sustaining this process. One major drawback in pursuing an elucidation of TR1's role in this process is that LLC1 cells do not have a parental, normal cell line for comparison. We therefore undertook a study of the knockdown of TR1 in DT cells, which represent an oncogenic *k-ras* cell line derived from parental, normal NIH 3T3 cells [27]. Interestingly, the morphological changes that resulted in DT cells following the targeted removal of TR1 were more characteristic of the parental NIH 3T3 cells than

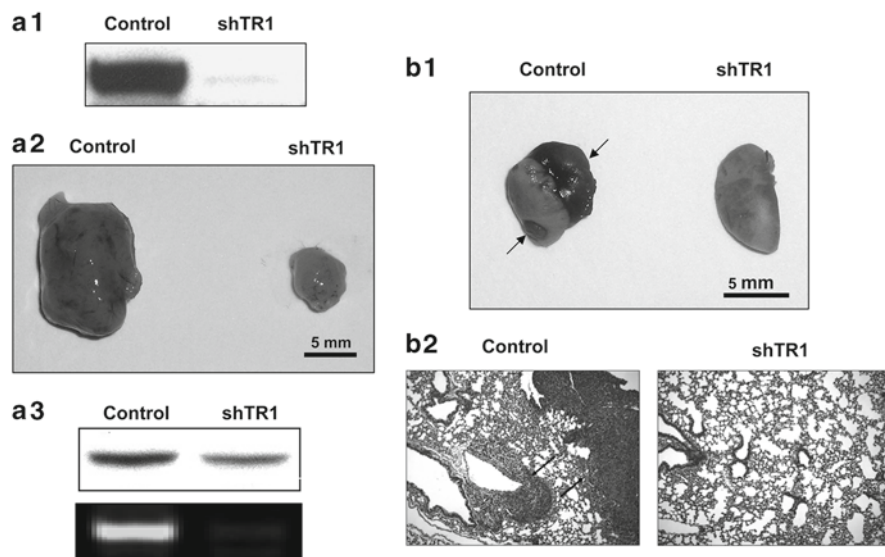


Fig. 25.1 Tumorigenicity and metastasis of TR1-deficient LLC1 cells. In **(a1)** western blot analysis of TR1 in LLC1 cells encoding the control or TR1 knockdown construct is shown. TR1 was more than 90% removed in knockdown cells. In **(a2)** tumors removed from mice that had been injected subcutaneously with LLC1 cells expressing either the control or TR1 knockdown construct are shown. Tumor size was much larger in mice injected with the control vector. In **(a3)** western blot analysis of TR1 levels that were present in the two tumors from **a2** (*upper panel*) and PCR analysis of genomic DNA isolated from these tumors (*lower panel*) are shown. TR1 is present in the smaller tumor (*upper panel*) as the TR1 knockdown vector is lost in the tumor from mice injected with the knockdown vector (*lower panel*). The data clearly show that both tumors express TR1 and that the knockdown vector is lost in the smaller tumor that resumes TR1 expression; and most importantly, that TR1 expression is essential for tumor growth. In **(b1)** tumor formation as a result of metastasis in the lung of the mouse injected with cells encoding the control vector and the normal appearing lung from the mouse injected with cells encoding the TR1 knockdown vector are shown. In **(b2)** tissue slices are shown from the lungs in **(b1)** that were analyzed for pathological changes; and the lung from the mouse injected with control vector manifested severe pathological abnormalities while the lung from the mouse injected with the knockdown vector appeared normal. See [26] for details. The figure was taken from [26] and modified slightly as shown here with permission from the *Journal of Biological Chemistry*. In the figure, shTR1 designates small hairpin RNA directed towards knocking down TR1 expression

DT cells transfected with the control vector. Compared to the TR1 overexpressing DT control cells, the corresponding TR1-deficient cells were found to have (i) lost their self-sufficiency growth properties, (ii) a defective progression in the S growth phase, and (iii) a decreased expression of DNA polymerase α , which is important in DNA replication [27]. Studies using DT TR1-deficient and sufficient cells and the corresponding parental (normal) NIH 3T3 cells, have provided considerable insight into the direct role of this selenoenzyme in many of the requirements governing the malignancy process and suggest possible novel avenues for inhibiting cancer.

25.3 Sep15

Sep15 was originally reported in 1998 as a 15 kDa selenium-containing protein in human T cells [29] and was subsequently shown to occur in a complex with UDP-glucose:glycoprotein glucosyltransferase (UGTR) [30]. UGTR is localized in the endoplasmic reticulum in mammalian cells and is involved in the quality control of protein folding [31]. The gene for Sep15 resides on chromosome 1p31 and contains five exons and four introns [32]. Recent studies on Sep15 have provided various insights into its structure and possible function. NMR structural analysis of Sep15 suggests a role in the reduction or isomerization of disulfide bonds of glycoprotein substrates of UGTR [32].

There are numerous lines of evidence suggesting that Sep15 has a role in cancer prevention. For example, earlier studies had shown that Sep15 exhibits redox properties [32], is structurally similar to the thioredoxin superfamily [33], belongs to the class of thioldisulfide oxidoreductase-like selenoproteins [33], and its gene exists on a chromosomal locus that is often deleted or mutated in human cancers [32, 34]. The highest levels of Sep15 expression were reported to occur in human and mouse liver, brain, testes, and prostate, but these levels were found to be reduced in hepatocarcinoma, lung cancer, and a prostate cancer cell line [32]. Furthermore, two polymorphic sites were found in *sep15* at nucleotide positions 811 (C/T) and 1125 (A/G) in the 3'UTR, and the latter polymorphism resides in the Sec Insertion Sequence (SECIS) element [29]. Interestingly, these two polymorphisms show different responses to selenium supplementation with regard to the efficiency of Sec incorporation into the growing polypeptide chain of Sep15 during translation [35]. Differences in frequencies of these two alleles were found to be associated with breast or head and neck tumors within African Americans and among different ethnic groups [36]. Recently, the A/T polymorphism in *sep15* was correlated with an increased risk of rectal cancer in Korean men [36].

There are additional studies that also suggest a role of Sep15 in cancer prevention. Malignant mesothelioma cells that were found to be sensitive to selenite toxicity have decreased Sep15 expression and further downregulating this protein by targeting its knockdown demonstrated that the cells were less sensitive to selenite [37]. In addition, this malignant cell line containing the A¹¹²⁵ polymorphic allele was also less sensitive to the effects of selenite than the corresponding cell line carrying the G¹¹²⁵ allele [38]. An increase in lung cancer risk was observed among smokers encoding a GG¹¹²⁵ or GA¹¹²⁵ genotype compared to those carrying an AA¹¹²⁵ genotype [38].

Many of the above studies suggest a role of Sep15 in cancer protection. However, other studies suggest a role of Sep15 in cancer promotion. The National Cancer Institute maintains a Developmental Therapeutics Program Database (<http://dtp.nci.nih.gov/mtweb/targetdata>) that screened 60 human tumor cell lines for molecular targets. An analysis of the data on these cell lines revealed an increase in Sep15 expression in colon cancer cell lines relative to other selenoproteins and to other cancers. These observations suggest that, by analogy to many other malignant cells, wherein TR1 is overexpressed and is used as a target for cancer therapy, Sep15

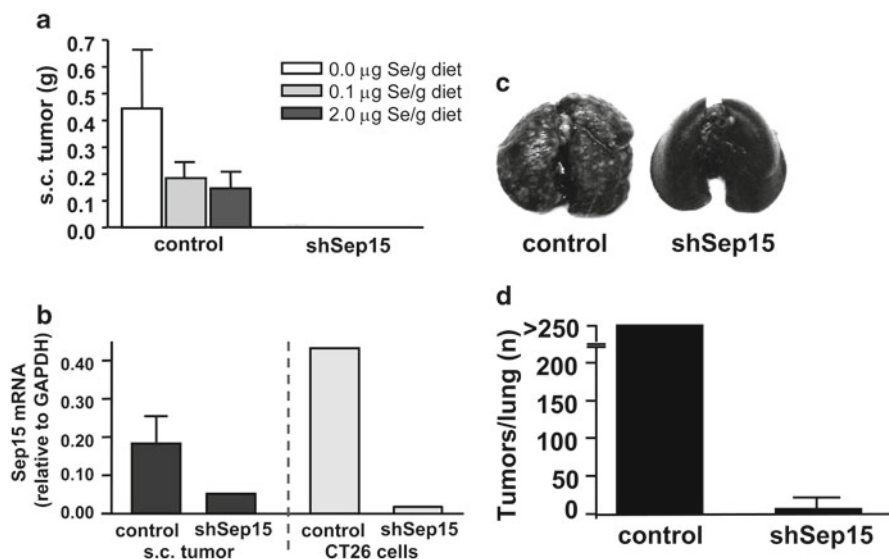


Fig. 25.2 Tumorigenicity and metastasis of Sep15-deficient CT26 cells. In (a) the weights of tumors removed from mice fed selenium-deficient (0 ppm selenium in the feed), selenium-adequate (0.1 ppm selenium) and selenium-enriched diets (2.0 ppm selenium) that were injected in the flank with the control or Sep15 knockdown vector are shown. Mice maintained on the selenium-deficient diet clearly had larger tumors. In (b) quantitative RT-PCR analysis of Sep15 mRNA from tumors of mice injected with control or Sep15 knockdown vector (*left two bars*) and mRNA from control and Sep15 deficient cells (*right two bars*) are shown. The amount of Sep15 knockdown vector relative to the amount of control vector is proportionally much higher in tumors formed in the flanks of mice than in Sep15 control and deficient cells. In (c) tumor formation as a result of metastasis in lungs of mice injected with control or Sep15-deficient cells. The tumors are shown in *white* on the surface of the lungs as a result of the black ink stain used for injection [39]. In (d) tumors on the surfaces of the lungs were counted and lungs with >250 lesions were designated as 250. The data are from three independent experiments. See [39] for details. The figure was reproduced from [39] with the permission of *Cancer Prevention Research*. In the figure, shSep15 designates small hairpin Sep15 RNA directed towards knocking down Sep15 and s.c. designates subcutaneous

might be a target for cancer therapy in colon cancer cells. We therefore undertook a study to evaluate the role of Sep15 in colon cancer. Sep15 mRNA was targeted for removal in a mouse colon cancer cell line, CT26, that had elevated Sep15 levels [39]. The resulting Sep15-deficient cells had a reduced growth rate and their ability to grow in soft agar was also reduced. Injection of the CT26 cells encoding the Sep15 knockdown vector into wild type, genetically compatible mice maintained on selenium-deficient, selenium-sufficient, or selenium-supplemented diets resulted in a large reduction in tumor number and size in mice injected subcutaneously compared to mice injected identically, but with control cells (i.e., those encoding the control vector). Fourteen of 15 mice injected with control cells developed tumors and the tumor size varied depending on the selenium level in the diet (Fig. 25.2a). Only one tumor was observed in mice injected with the Sep15 knockdown vector,

and an examination of this tumor for the presence of the knockdown vector showed that it had been lost (Fig. 25.2b). Similar to the experiments carried out with the knockdown of TR1 in LLC1 cells described above, wherein tumor development was found to be dependent on TR1 expression, it also appeared that tumor development in the CT26 cells was dependent on Sep15 expression [39]. The Sep15 deficient, CT26 cell line also manifested a dramatically reduced metastatic ability in forming tumors in the lungs of mice compared to control cells when the two cell lines were injected into mice intravenously (Fig. 25.2c, d) [39].

To elucidate the possible means of how Sep15-deficient CT26 cells can have such pronounced effects on colon cancer cell growth, anchorage-independent cell growth in soft agar, tumorigenicity and metastasis, these Sep15 knockdown cells were compared to the corresponding Sep15-expressing CT26 cells using microarray analysis [39]. Those genes that were the most significantly up- or downregulated indicated primarily biological functions related to cancer, and cellular growth and proliferation as the main molecular and cellular functions affected. The gene with the highest upregulation (13.5-fold increase in Sep15-deficient cells) encoded for the cyclin B1-interacting protein 1, which was subsequently validated using real-time RT-PCR. Fluorescence-activated cell sorting analysis confirmed the G₂-M cell arrest in Sep15-deficient cells suggested by microarray analysis. It appeared that Sep15 loss resulted in reversal of the original cancer phenotype of the murine colon cancer cells that was mediated, at least in part, by upregulation of cell cycle-related genes, and likely resulting in subsequent G₂-M cell cycle arrest.

Currently, we are investigating the effects of Sep15 loss in colon cancer in vivo, quantitating chemically induced aberrant crypt foci development in colonic epithelia of Sep15 knockout mice. Preliminary results indicate that lack of Sep15 is protective against formation of aberrant crypt foci (PA Tsuji, BA Carlson, S Naranjo-Suarez, M-H Yoo, X-M Xu, DE Fomenko, VN Gladyshev, DL Hatfield, CD Davis, submitted for publication). Furthermore, a cytokine-regulated GTPase was highly upregulated in Sep15 knockout animals and its possible link to Sep15 is being elucidated further.

25.4 Conclusions and Other Roles of Selenium in the Cancer Process

It is most interesting that the malignancy of a lung cancer mouse cell line, LLC1, appeared to be dependent on TR1 expression, and similarly, a mouse colon cancer cell line, CT26, appeared to be dependent on Sep15 expression. If, indeed, these cell lines are being driven by two different selenoproteins, which also have been shown to have roles in cancer prevention, a question arises as to how TR1 and Sep15 can have such overall opposing roles in their behavior. One seemingly plausible explanation is that their roles in cancer and normal cells are much the same in that they help maintain a balanced redox system. However, the burdens of oxidative stress are most certainly far greater, and the metabolic demands far broader, in maintaining an

efficient redox system in cancer cells than in normal cells. Hence, the higher levels of expression of these two selenoproteins in their respective malignant cell lines are likely a reflection of greater demands for the services of these two selenoproteins that may include expanded roles in cancer. In support of such a proposal, we have recently shown that TR1 assumes new roles that appear to be in large part independent of its known role in reducing thioredoxin in DT cells suffering from selenite toxicity as compared to the parental, NIH 3T3 cell line or to DT cells not exposed to selenite (R Tobe, M-H Yoo, N Fradejas Villar, BA Carlson, S Calvo, VN Gladyshev, DL Hatfield, submitted for publication).

To our knowledge, there are only two studies showing that selenium deficiency can also play a role in cancer prevention. The progression of peritoneal plasmacytoma (PCT) in mice was found to be dependent on chronic peritoneal inflammation following injection with pristane [40]. Interestingly, virtually no PCT occurred in selenium-deficient mice injected with pristane, whereas about 40% of the mice fed selenium-adequate diets developed PCT. In another study, liver tumor formation was inhibited in TGF α /c-Myc transgenic mice, which are well known to develop hepatomas [41], maintained on a selenium-deficient diet compared to mice maintained on the same diet but supplemented with 0.1 or 0.4 ppm selenium [42]. These studies are surprising, as are those showing that specific selenoproteins have roles in promoting cancer, in that selenium has long been heralded as a cancer preventive. It is of further interest that the latter study involving TGF α /c-Myc transgenic mice also demonstrated that mice fed a diet supplemented with 2.25 ppm selenium appeared to be protected from liver cancer compared to mice maintained on the other two selenium diets. This study showed that a selenium-deficient and a highly enriched selenium diet reduced liver tumor formation. Very importantly, these studies reveal the complexities and the consequences involved in an imbalance of the selenium population (and selenium, small molecular weight selenocompounds and/or selenoproteins may be involved) on the resulting affected cells. They also provide clear illustrations of how insufficient our knowledge is regarding the biology of selenium. Furthermore, the studies discussed in this chapter suggest how extremely important it is not to pursue human clinical trials involving selenium until we learn far more about the underlying selenium metabolic pathways and how they act in promoting as well as preventing cancer.

Acknowledgements This work was supported by the National Institutes of Health NCI Intramural Research Program and the Center for Cancer Research (to D.L.H.) and by National Institutes of Health Grants (to V.N.G.).

References

1. Hatfield DL, Berry MJ, Gladyshev VN (2006) Selenium: its molecular biology and role in human health. Springer Science + Business Media LLC, New York
2. Hatfield DL, Berry MJ, Gladyshev VN (2011) Selenium: its molecular biology and role in human health. Springer Science + Business Media LLC, New York

3. Ip C, Dong Y, Ganther HE (2002) *Cancer Metastasis Rev* 21:281
4. Diwadkar-Navsariwala V, Diamond AM (2004) *J Nutr* 134:2899
5. Rayman MP (2005) *Proc Nutr Soc* 64:527
6. Lü J, Jiang C (2005) *Antioxid Redox Signal* 7:1715
7. Davis CD, Irons R (2005) *Curr Nutr Food Sci* 1:201
8. Hatfield DL, Xu XM, Carlson BA et al (2006) *Prog Nucl Acid Res Mol Biol* 81:97
9. Brigelius-Flohé R (2008) *Chem Biodivers* 5:389
10. Yang JG, Hill KE, Burk RF (1989) *J Nutr* 119:1010
11. Behne D, Kyriakopoulos A, Gessner H et al (1992) *J Nutr* 122:1542
12. Irons R, Carlson BA, Hatfield DL et al (2006) *J Nutr* 136:1311
13. Kryukov GV, Castellano S, Novoselov SV et al (2003) *Science* 300:1439
14. Arnér ES (2009) *Biochim Biophys Acta* 1790:495
15. Maiorino M, Scapin M, Ursini F et al (2003) *J Biol Chem* 278:34286
16. Burk RF, Hill KE (2009) *Biochim Biophys Acta* 1790:1441
17. Merrill GF, Dowell P, Pearson GD (1999) *Cancer Res* 59:3175
18. Moos PJ, Edes K, Cassidy P et al (2003) *J Biol Chem* 278:745
19. Seemann S, Hainaut P (2005) *Oncogene* 24:3853
20. Rundlöf AK, Arnér ES (2004) *Antioxid Redox Signal* 6:41
21. Biaglow JE, Miller RA (2005) *Cancer Biol Ther* 4:6
22. Arnér ES, Holmgren A (2006) *Semin Cancer Biol* 16:420
23. Fujino G, Noguchi T, Takeda K et al (2006) *Semin Cancer Biol* 16:427
24. Nguyen P, Awwad RT, Smart DD et al (2006) *Cancer Lett* 236:164
25. Lu J, Chew EH, Holmgren A (2007) *Proc Natl Acad Sci USA* 104:12288
26. Yoo MH, Xu XM, Carlson BA et al (2006) *J Biol Chem* 281:13005
27. Yoo MH, Xu XM, Carlson BA et al (2007) *PLoS One* 2:e1112
28. Hatfield DL, Yoo MH, Carlson BA et al (2009) *Biochim Biophys Acta* 1790:1541
29. Parodi AJ (2000) *Biochem J* 348:1
30. Korotkov KV, Kumaraswamy E, Zhou Y et al (2001) *J Biol Chem* 276:15330
31. Ferguson AD, Labunskyy VM, Fomenko DE et al (2006) *J Biol Chem* 281:3536
32. Kumaraswamy E, Malykh A, Korotkov KV et al (2000) *J Biol Chem* 275:35540
33. Labunskyy VM, Hatfield DL, Gladyshev VN (2007) *IUBMB Life* 59:1
34. Nasr MA, Hu YJ, Diamond AM (2004) *Cancer Ther* 1:293
35. Hu YJ, Korotkov KV, Mehta R et al (2001) *Cancer Res* 61:2307
36. Sutherland A, Kim DH, Relton C et al (2010) *Genes Nutr* 5:215
37. Apostolou S, Klein JO, Mitsuuchi Y et al (2004) *Oncogene* 23:5032
38. Jablonska E, Gromadzinska J, Sobala W et al (2008) *Eur J Nutr* 47:47
39. Irons R, Tsuji PA, Carlson BA et al (2010) *Cancer Prev Res* 3:630
40. Felix K, Gerstmeier S, Kyriakopoulos A et al (2004) *Cancer Res* 64:2910
41. Santoni-Rugiu E, Nagy P, Jensen MR et al (1996) *Am J Pathol* 149:407
42. Novoselov SV, Calvisi DF, Labunskyy VM et al (2005) *Oncogene* 24:8003